



## Comparison of Creatine and Glutamine supplementation consumption along with resistance exercise on the level of ALP in female mice

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### ABSTRACT

In recent years, in order to improve power, speed, the increase in the volume of the musculature, preventing sports injuries and maintain the muscle performance athletes use from different resistance exercises and food supplements. In this regard, present study has been conducted with the aim of comparison the influence of an 8 week period consumption of creatine ( $2 \text{ gr kg}^{-1} \text{ day}^{-1}$  in 1<sup>st</sup> week and  $0.48 \text{ gr kg}^{-1} \text{ day}^{-1}$  during 2<sup>nd</sup> to 8<sup>th</sup> weeks) and glutamine ( $1 \text{ gr kg}^{-1} \text{ day}^{-1}$  from first to eighth weeks) along with resistance exercise on the weight and level of ALP of female mice. This experimental study was done on 80 Small adult female mice of Surrey species ( $28 \pm 5$  gram). The animals were randomly divided into 8 main groups of: resistance exercise, resistance exercise + creatine, resistance exercise + glutamine, resistance exercise + glutamine + creatine, creatine, glutamine, creatine + glutamine and control groups ( $N= 10$ ). Resistance exercise (5 days a week) was including: climbing (4 sets, 5 times repetition with two minutes rest between the sets) from a ladder (with the height of one meter and including 26 steps) and bearing 30 percent of the weight of the Mouse body (hanging from tail) in the first week and the increasing it up to 200 percent of body weight till the last week of the experiment. During 48 hours after the last practice session of resistance exercise, the blood sample was taken and the the level of ALP has been measured. The results showed that the level of ALP enzyme in groups supplemented with creatine+ glutamine (combined with each other) and underwent the resistance exercise had been increased in comparison with the control group ( $144.3 \pm 15.86$  in comparison with  $234.7 \pm 25.69 \text{ U.L-I}$ ;  $P < 0.05$ ). The results of this research indicate an increase in the level of ALP enzyme in the liver of mice under effect of the resistance exercise and consumption of creatine-glutamine supplementation and it showed that consumption of glutamine supplementation along with resistance exercise has the most influence upon the animals' weight. Therefore these results suggest more importance of them and more studies are necessary to be done.

**Key words:** ALP, liver, creatine, glutamine, resistance exercise, female mice.

### INTRODUCTION

One of the issues, that athletes since long time ago have looked for, is improving the sport performances. In recent years many food supplements have drawn the attention of researchers and their inquiring mind have been directed toward the studying different influences of supplements on muscular power, body shape and other physiological mechanisms. A wide range of researches have investigated the effect of food supplements on the performance of sports. The other methods, by which athletes use for increasing and promoting physical fitness level, are resistance exercises. In general, Athletes use different resistance exercises in order to increase the power, the speed, enhancing the muscle volume, preventing sports injuries and maintaining muscle performance[1,2].

On the other hand in recent years along with athletic exercises, consumption of food supplements also has become popular and almost all athletes in their life sports, despite a lack of knowledge about the influence of these supplementations, have used one or few of them [3]. Although researches have shown that many of these

supplements have no effect on performance of people, however, it has been proven that one of the most effective diet supplements existing for the athletes is creatine supplement. Therefore, it can be said that creatine is probably the most useful sports supplement for improving athletes' performance. However, still there are some worries about the harmful effect of short term and long term consumption of the creatine on the health [4]. Also, a lack of significant influence of glutamine supplements on some enzymes such as creatine kinase has been reported [5].

Therefore, study in this field will lead to achieve better goals in a sport performance. At the end, liver enzymes (including ALP) due to being influenced with resistance exercises and consumption of the diet supplements are utilized as the index for detection effect of food supplements and resistance exercises [6,7,8]. In a study done by Waldron et al, it was detected that consuming creatine supplement during exercise has no meaningful effect on hepatic disorder (amount of ALP enzyme activity) [9]. In the other hand, Saki et al also investigated the impact of supplement consumption on the level of various enzymes in athletes whom were doing resistance exercises. Their results showed that level of ALP has elevated [10].

With regard to the above cases and existing contradictions about the impact of supplements and athletic exercises on the health and weight of athletes and especially combination effect of these supplements on the liver, that has an important and fundamental role in many body functions, Therefore the present research with the aim of determining influence of resistance exercises and creatine and glutamine supplements consumption on the levels of ALP and weight of laboratory small female mice was conducted.

### EXPERIMENTAL SECTION

In this research, all the cares of the animals were performed based on ethical guideline in animal experiment research of Brazil (COBEA) and animal scientific research committee of university of Vale do Paraíba ( ethical code of present research : 493059).

#### -Experimental design

The present investigation is an experimental study and was done on 80 laboratory adult female mouse three month old ( $28.00 \pm 5.0$  gram) which were kept separately ( $22 \pm 2$  degrees centigrade, with air moisture of the  $50 \pm 5$  % and the cycle of light to darkness 12:12 hours). Water and food was freely available. The study was done for eight weeks and animals were randomly separated to 8 Groups (N=10):resistance exercise, resistance exercise+ creatine, resistance exercise+ glutamine, resistance exercise+ glutamine+ creatine, creatine, glutamine, creatine+ glutamine and control groups.

#### -Resistance exercise

Climbing from a ladder with the height of one meter (with 26 steps, with 2cm distance and slope of 85 degrees) and connection of sinker to the tail of mouse as a resistance exercise were used. Mice got familiar with the exercise for one week. After acquaintance, resistance exercises got started using cylinder contained sinker which was closed with an adhesive to the tail of mice (2-3 cm from the proximal end). After closing the weight to the tail of small laboratory female mice, they encouraged to climb. In the third week in which mice were adapted to exercise protocol, in the beginning for adaptation we used encouragement with food and touch, but after a few weeks the mice used to do them and immediately when they placed in the foot of ladder began to climb. In the first week the amount of closed traction was up to 30 percent's of weight of their body that was gradually increased and it reached about 200 percent's of their weight in the last week (table 1). Resistance exercises were including five days in a week containing 4 sets, 5 time repetitions with one minute rest between repetitions and two minutes between the sets for eight weeks.

Table1– The resistance exercise program in 4 sets of 5 repetitions on the one meter ladder with 26 steps and 2cm distance

week	I	II	III	IV	V	VI	VII	VIII
Load (percentage of the body weight)	30	70-80	100	120-130	140-150	170-175	180-190	200

#### -Supplements

Supplements either alone, along with resistance exercise and or in combination with each other were given to mice as the following. Animals received the supplements via gavage.

#### -Creatine supplement

The creatine supplement was started along with resistance exercise. Dosage used for creatine was  $2 \text{ gr.kg}^{-1}.\text{day}^{-1}$  (loading stage) in the first week and  $0.48 \text{ gr.kg}^{-1}.\text{day}^{-1}$  during 2nd to 8th weeks (maintenance stage). The dose was administered 30 minutes before exercise resistance to the animals.

**- Glutamine supplement**

The glutamine supplement was begun along with resistance exercise. Dosage used for glutamine was  $1 \text{ gr.kg}^{-1}.\text{day}^{-1}$  from in all eight weeks. The considered dose was given to the animals 60 minutes after resistance exercise (or after feeding with creatine supplement to groups without resistance exercise).

**- Enzyme level measurement**

Forty-eight hours after the last resistance exercise, the animals were killed and blood sample was taken. In order to separate the serum from blood cells, obtained blood samples were centrifuged at temperature of 4 degrees celcius with the speed of 5000 round per minute (rpm) for 10 minutes. The measurement of activity levels of Alkaline phosphatase (ALP) were performed with ELISA method ( Enzyme-linked immunosorbent) and with the utilizing the kits of HANGZHOU EASTBIOPHARM company with the help of the auto- analyser RA-1000 made of Technicom company of the US.

**-Statistical method**

We applied kolmogorov-smirnov test for determining the normality of data. Of one-way analysis of variance and LSD post hoc test for comparison of difference among groups were used. For analysis of data SPSS software version 19 (version 19, SPSS Inc, Chicago, IL) was used and significant level was considered  $P < 0.05$  Also the results were presented based on the mean and Standard Deviation.

**RESULTS AND DISCUSSION**

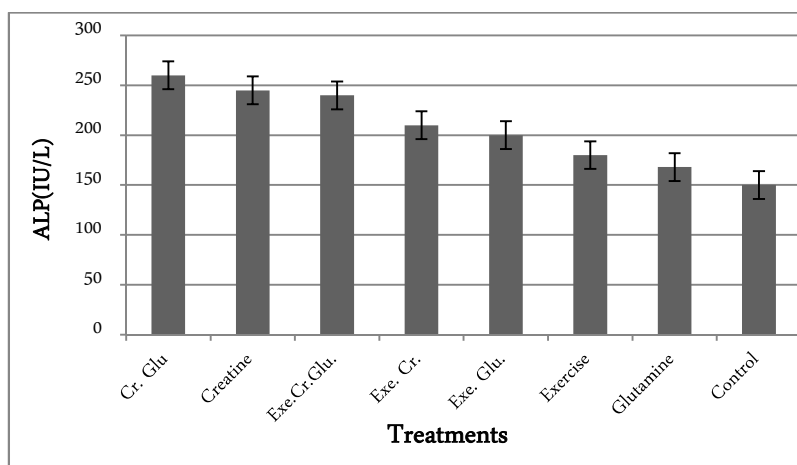
According to the given results in the table number2, kolmogorov-smirnov test showed that data correlated to ALP liver enzyme and weight of the mice followed a normal distribution ( $P > 0.05$ ). Moreover, data have had a homogeneous variance ( $P > 0.05$ ).

**Table2-the test results of kolmogorov-smirnov test and homogeneity of variances for ALP liver enzyme and weightof mice**

Variable	kolmogorov-smirnov	significance level	homogeneity of variances
ALP enzyme(IU/L)	0.66	0.76	0.33
Weightof mice(gram)	0.71	0.59	0.41

**- ALP enzyme of miceliver**

Corresponding to the results, one-way analysis of variance showed that the amount of ALP enzyme of mice liver of control group in comparison with mice groups underwent different treatments (glutamine, creatine, creatine+ glutamine, exercise + creatine, exercise + glutamine, exercise + creatine + glutamine and exercise) had a meaningful difference ( $P < 0.05$ ). So that, according to LSD test results, in mice which had consumed the creatine and glutamine supplementations along with resistance exercise and group under resistance exercise, in comparison with the control group the levels of liver enzyme ALP had increased (Figure 1).



**Figure1- comparison of the level of ALP (IU/L)enzyme of control group with7 applied groups using the LSD test (Cr.: Creatine, Glu.: Glutamine, Exe.: Exercise)**

Based on the results of this research, 8 week resistance exercise and consumption of supplementations has a significant influence on the level of the ALP liver enzyme and body weight of the mice. The other researchers have achieved to the similar results as our study [11]. Also the results of this study showed that resistance exercise

doesn't cause a meaningful change in the amount of ALP liver enzymes in patients with liver disorder and it could be one of the distinctions of exercise resistance in comparison with supplements such as creatine. Barani and his colleagues also stated that resistance exercise with a reduction in the level of ALP enzyme and an improvement in physical fitness parameters can be effective in recovery of patients with fatty liver [12,13]. Also Bemben et al have found the similar results and reported that resistance exercise doesn't change the level of ALP meaningfully [14]. Additionally based on the results of this research, the amount of ALP enzyme in the liver of rats under the influence of glutamine supplementation consumption in comparison with the control was without a significant difference. Faokhshahi Nia et al also in a study in order to determine the effect of glutamine supplementation on the severity of perceived pain stated that this supplement has no effect on the Index of muscle damage and causes a reduction in muscle pain created with exercises [14].

In the field of how glutamine affects, Pithon and colleagues stated that glutamine in improving and control of inflammatory processes including activity of neutrophils has an effective role in the increase of host defense mechanism and lead to the decrease of the inflammatory periods and the fibrous death [15]. Cruzat et al about the influence of glutamine supplement consumption on indices of muscle damage mentioned that consumption of glutamine supplementation decrease the release of enzyme immediately after exercise protocol [16]. So this is one of the reasons that this glutamine supplement is important for athletes. The results of Dabidi et al' study indicates a lack of meaningful influence of glutamine supplement on some of immune indices due to sport activities [17]. On the other hand creatine supplement consumption has caused the significant increase in the amount of liver enzymes of ALP in liver of mice in comparison with both control group and other studied groups. Researchers in this field have studied the liver enzyme changes and have achieved to various results. In the sense that creatine probably can prevent the increase in the activity of these enzymes [18,19]. While, some of the researches have reported the role of creatine in the increase of these enzyme, in other it is indicated that consumption of creatine on the level of Serum enzymes is ineffective[20].

The other findings of this study was that effect of a combination of resistance exercise along with creatine and with glutamine + creatine though caused a significant increase in amount of liver enzyme of ALP compared to the control group but it had less effect compared to mice that had consumed creatine and creatine + glutamine.

Therefore generally, based on finding of this part of the research it can be concluded that if resistance exercise is done along with consuming different supplementations, to a great extent causes an adjustment in the amount of release of ALP enzyme.

Robinson et al also stated that simultaneous use of combination of creatine supplement with athletic exercises will not increase the liver enzymes significantly [20].

According the results of this study, if a resistance exercise is done parallel with consumption of supplement, to a large extent it will decrease the amount of ALP enzyme in the liver. Bashiri and his colleagues also expressed that using a combination of resistance exercise and consumption of creatine monohydrate as the index of liver Cell Damage, there is no likely an undesirable increase in liver enzymes [19].

## CONCLUSION

Generally based on the findings of this study, weight of mice using supplements and resistance exercise in contrast to impact of the eight groups is to a large extent influenced by the amount of the secretion of ALP. In the other words, the consumption of glutamine supplement with resistance exercise had the least influence in secretion of ALP of liver and the highest influence on the weight of the mice body.

But consumption of creatine supplement had the highest influence on the secretion of the ALP and the least effect on the weight of of the mice body. Therefore it may be possible to determine the influence of supplements on the body weight with detecting the amount of the secretion liver enzymes. Of course to achieve the definite answer it is needed more studies to be done. At the end results of this investigation indicates the distinction between influence of creatine and glutamine supplement. Because glutamine had less influence in secretion of ALP enzyme and more important role in the increase in weight of the body and its effects was almost parallel and close with resistance exercise. Of course it should be mentioned that a combination of supplementation of glutamine + creatine has much effect on liver enzymes ALP secretion, though this can be related to some extent to the effect of the creatine, however more investigations are mandatory.

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